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A Nosocomial Outbreak Due to *Enterobacter cloacae* Strains with the *E. hormaechei* Genotype in Patients Treated with Fluoroquinolones

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During a 7-month period, we isolated 21 highly fluoroquinolone-resistant *Enterobacter cloacae* strains in units from two hospitals in Marseille, France. Random amplification of polymorphic DNA showed clonal identity between isolates which, furthermore, presented the *Enterobacter hormaechei* genotype on DNA-DNA hybridization. The emergence of this clone was observed only in patients treated with fluoroquinolones.

Clinically, *Enterobacter cloacae* and *Enterobacter aerogenes* are the most common species in the genus *Enterobacter*. For 10 years, the two species have been increasingly considered as causing infections in hospitalized patients (8, 9, 18). They are characterized by resistance to broad-spectrum beta-lactam agents by producing extended-spectrum β-lactamase and inducible chromosome-encoded cephalosporinase (1, 14, 19). Current outbreaks caused by *E. cloacae* are specifically correlated with the heavy use of extended-spectrum beta-lactams or aminoglycosides (11, 19). During 7 months, we noted a surprisingly frequent isolation rate in the laboratory of *E. cloacae* strains phenotypically similar to each other and highly resistant to fluoroquinolones. To determine whether they were epidemiologically related, typical emergent isolates were compared by the random amplification of polymorphic DNA (RAPD) (20), which has been described as a useful tool for the epidemiological typing of *Enterobacter* spp. (5, 6, 10, 11).

Between November 1994 and May 1995, we collected 21 clinical *E. cloacae* isolates from 15 infected or colonized patients in seven units in two hospitals in Marseille, France (Table 1). Patients 1 to 14 were hospitalized in different units of a same hospital, and patient 15 was hospitalized in a nephrology unit of the other hospital. None of these patients was transferred from one hospital to the other during the study period. The locations of the patients concerned raised the question of patient-to-patient transmission.

An epidemiological investigation led us to conclude that no medical relationships were evident between patients from different units. Except for a common food service, the two hospitals had independent medical staffs and equipment.

The strains were isolated on media from routine clinical specimens: bronchial secretions, urine samples, closed cavity drainage specimens, catheters, and wound swabs. From one to three strains were studied per patient, depending on the duration of hospitalization. All isolates were considered nosocomially acquired because samples were taken at least 48 h after hospitalization. Four reference strains, the type strain (ATCC 49162) and three strains obtained from the Collection of Institut Pasteur (Paris, France) (strains 4911-84, 4104-83, and 4521-86), were analyzed. For controls for the RAPD typing technique, we used two *E. cloacae* and two *E. aerogenes* strains isolated during the same period from patients in the two hospitals. All strains were identified by the API 20 E identification system (bioMérieux, Marcy L'Etoile, France) according to the manufacturer's instructions. The strips were incubated at 30°C for 1 day. Carbon source utilization tests were done with Biotype-100-carbon source strips (bioMérieux) (2). According to the manufacturer's guidelines, the strips were inoculated with Biotype 1 assimilation medium and incubated at 30°C. Readings were done on days 2 and 4. Susceptibilities to 44 antimicrobial agents were determined by the standard disk diffusion method on Mueller-Hinton agar (bioMérieux) (16). The isolates were investigated for molecular typing by RAPD as previously described (6). We used primers AP12H (5′-CGGCCC CTGT-3′) and HLW74 (5′-ACGTATCTGC-3′). As in a previous study (21), strains were considered different if their profiles differed by two or more bands. For DNA-DNA hybridization, 2-μg samples of native DNAs were labelled by nick translation with a nick translation kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) by using tritium-labelled nucleotides (Amersham International, Amersham, England). The procedures for the hybridization experiments in which the S1 nuclease-trichloracetic acid method was used were performed as described previously (3, 13). Experiments were performed twice to verify reproducibility. Both DNAs were labelled and hybridized with unlabelled DNA to avoid errors due to possible differences in genome sizes.

The 21 clinical strains presented the same API numerical profile 1305573, corresponding to *Enterobacter intermedius* in the 1990 edition of the analytical profile index and *E. cloacae* in the 1994 edition. However, with the Biotype-100-carbon source strips, they presented the typical pattern for *E. hormaechei*, with the number of positive characters ranging from 56 to 60. These differences were probably due to different adaptations of the isolates in patients. The *E. hormaechei* strains studied by O'Hara et al. (17), as well as the reference strains obtained from the Collection of Institut Pasteur, were lysine decarboxylase and gelatinase negative, generally ornithine decarboxylase, arginine dihydrolase, and urease positive, and fermented sucrose and L-rhamnose but not sorbitol and melibiose. However, the strains isolated during this outbreak presented a few characteristics atypical of *E. hormaechei*: they were sorbitol positive and melibiose positive. On DNA hybridization, they presented a homology of 80% with the *E. hor-*

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TABLE 1. Treatment, clinical, and bacteriological data for patients

Patient	Ward[a]	Hospitalization procedure, disease, or condition	First bacterium isolated	Antibiotic therapy	Treatment start date (day/mo/yr)	Date (day/mo/yr) of isolation of *E. hormaechei*	Delay between start of treatment and isolation of *E. hormaechei* (no. of days)
1	ICU	Aortic dissection	*E. aerogenes*	Pefloxacin	01/09/94	13/09/94	12
2	ICU	Acute respiratory failure	*E. aerogenes*	Ciprofloxacin	02/09/94	14/09/94	12
3	Surgery	Spondylitis		Pefloxacin	10/08/94	13/10/94	64
4	Cardiology	Cardiac failure	*E. aerogenes*	Pefloxacin	29/10/94	02/11/94	18
5	Nephrology 1	Acute renal failure	*E. cloacae*	Pefloxacin	28/10/94	02/11/94	6
6	Nephrology 1	Acute renal failure	*E. aerogenes*	Pefloxacin	01/11/94	04/11/94	3
7	ICU	Acute respiratory failure	*E. cloacae*	Pefloxacin	30/10/94	12/11/94	13
8	Neurology	Inhalation pneumonia		Ciprofloxacin	22/11/94	26/11/94	4
9	Cardiology	Mitral surgery	*E. aerogenes*	Sparfloxacin	14/11/94	26/11/94	12
10	Neurology	Pneumonia		Pefloxacin	10/12/94	13/12/94	3
11	Gastroenterology	Mesenteric infarctus	*E. cloacae*	Ciprofloxacin	10/03/95	15/03/95	5
12	ICU	Thymoma	*E. aerogenes*	Ciprofloxacin	15/05/95	24/05/95	9
13	ICU	Acute respiratory failure	*E. cloacae*	Pefloxacin	15/04/95	26/05/95	11
14	Nephrology 1	Acute renal failure	*E. coli*	Pefloxacin	01/06/95	04/06/95	3
15	Nephrology 2	Acute renal failure	*E. coli*	Norfloxacin	01/06/95	08/06/95	7

[a] ICU, intensive care unit.

maechei type strain. The 21 clinical *E. cloacae* isolates with the *E. hormaechei* genotype presented identical susceptibility patterns (Table 2), an inducible chromosomal cephalosporinase, and were susceptible to the majority of extended-spectrum cephalosporins and to all aminoglycosides. All the isolates were identically highly resistant to the four fluoroquinolones tested (pefloxacin, ofloxacin, norfloxacin, and ciprofloxacin), with MICs ranging from 256 to 1,024 mg/liter. The patients concerned were treated by fluoroquinolones at least 3 days before the appearance of *E. cloacae* with the *E. hormaechei* genotype (Table 1). One patient was treated for 64 days with pefloxacin for spondylitis without bacterial isolation. All patients except for three were treated for infection due to *Enterobacter* spp. or *E. coli*. The others were treated by a fluoroquinolone for an infection without isolation of bacteria. On RAPD testing, all clinical isolates presented identical profiles with the two primers and were different from control or reference strains (Fig. 1).

E. cloacae was recently found to be genetically heterogeneous, and five DNA groups were evidenced (12). *E. hormaechei* fell in *E. cloacae* DNA group 3. The biotype 100 database for members of the family *Enterobacteriaceae* was updated to take these DNA groups into consideration. From these results, we believe that these infections were due to the dissemination of a clone of *E. cloacae* isolates with the *E. hormaechei* genotype characterized by high resistance to fluoroquinolones after fluoroquinolone treatment, as observed after norfloxacin treatment in a previous study (7). The isolates are genotypically identical to each other and belong to the genotype *E. hormaechei*. No similar isolate was identified in a retrospective study of strains isolated in our laboratory since 1990. The origin of this clone is unknown, since no common source was found. An

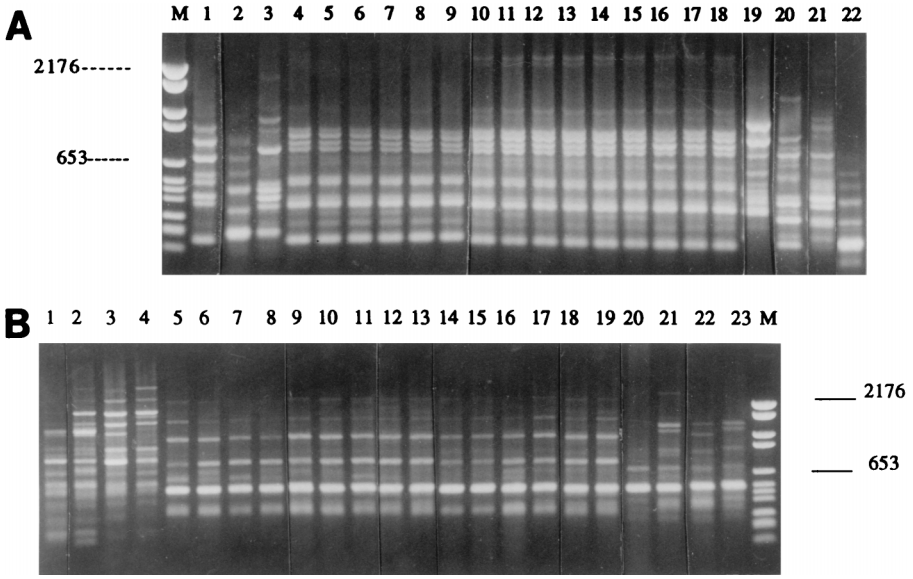

FIG. 1. Representative RAPD fingerprints obtained with primer AP12H (A) or HLW74 (B). (A) Lanes 1 and 2, control *E. cloacae* isolates; lane 3, control *E. aerogenes* isolate; lanes 4 to 18, 15 of the 21 clinical *E. cloacae* isolates; lanes 19 to 22, reference *E. hormaechei* strains. (B) Lanes 1 and 2, control *E. cloacae* isolates; lanes 3 and 4, *E. aerogenes* isolates; lanes 5 to 19, 15 of the 21 clinical *E. cloacae* isolates; lanes 20 to 23, reference *E. hormaechei* strains. Lanes M, size marker VI. Numbers to the left and right indicate size in base pairs.

TABLE 2. Comparison of antibiotic susceptibility patterns of *E. hormaechei* reference strains and *E. cloacae* clinical isolates

Antibiotic	Antibiotic susceptibility[a]	
	E. hormaechei reference strains (including the type strain)	*E. cloacae* clinical isolates
Penicillin G	R	R
Piperacillin	S	S
Imipenem	S	S
Cephalothin	R	R
Cefoxitin	R	R
Cefotaxime	S	S
Ceftriaxone	S	S
Ceftazidime	S	S
Latamoxef	R	R
Gentamicin	S	S
Tobramycin	S	S
Netilmicin	S	S
Amikacin	S	S
Cotrimoxazole	S	S
Pefloxacin	S	R
Ofloxacin	S	R
Ciprofloxacin	S	R
Fosfomycin	S	S

[a] R, resistant; S, susceptible.

epidemiological investigation was performed, but no relationships between patients except for a common food service could be demonstrated. As observed for *Stenotrophomonas maltophilia* with imipenem (4), these clones of *E. cloacae* emerged specifically with fluoroquinolone treatment; *E. hormaechei* was not isolated without fluoroquinolone treatment. A few months later, we observed a decrease in the *Enterobacter* species isolation after actions taken by the medical staffs of the two hospitals, in order to reduce the prescription of fluoroquinolones. *E. cloacae* strains with the *E. hormaechei* genotype have the pecularity of being susceptible to extended-spectrum cephalosporines, which points to the existence of porins. The mechanism of resistance to quinolones is probably an efflux mechanism or mutations of the region of the *gyrA* gene that determines resistance to quinolones, as observed for *Escherichia coli* (15). Most of the *E. hormaechei* strains studied by O'Hara et al. were isolated from clinical samples (17). To our knowledge, no *E. hormaechei* outbreak had been reported before.

REFERENCES

1. **Acolet, D., Z. Ahmet, E. Houang, R. Hurley, and M. E. Kaufmann.** 1994. *Enterobacter cloacae* in a neonatal intensive care unit: account of an outbreak and its relationship to use of third-generation cephalosporins. J. Hosp. Infect. **28:**273–286.
2. **Bouvet, O. M. M., P. A. D. Grimont, C. Richard, E. Aldova, O. Hausner, and M. Gabrhelova.** 1985. *Budvicia aquatica* gen. nov., sp. nov.: a hydrogen sulfide-producing member of the *Enterobacteriaceae*. Int. J. Syst. Bacteriol. **35:**60–64.
3. **Brenner, D. J., A. C. McWhorter, J. K. L. Knudson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. **15:**1133–1140.
4. **Davin-Regli, A., C. Bollet, J. P. Auffray, P. Saux, and P. De Micco.** 1996. Use of random amplified polymorphic DNA for epidemiological typing of *Stenotrophomonas maltophilia*. J. Hosp. Infect. **32:**39–50.
5. **Davin-Regli, A., P. Saux, C. Bollet, F. Gouin, and P. de Micco.** 1995. Investigation of outbreaks of *Enterobacter aerogenes* colonisation and infection in intensive care units by random amplification of polymorphic DNA. J. Med. Microbiol. **44:**89–98.
6. **Davin-Regli, A., D. Monnet, P. Saux, C. Bosi, R. Charrel, A. Barthelemy, and C. Bollet.** 1996. Molecular epidemiology of *Enterobacter aerogenes* acquisition: one-year prospective study in two intensive care units. J. Clin. Microbiol. **34:**1474–1480.
7. **Dupeyron, C., N. Mangeney, L. Sedrati, B. Campillo, P. Fouet, and G. Leluan.** 1994. Rapid emergence of quinolone resistance in cirrhotic patients treated with norfloxacin to prevent spontaneous bacterial peritonitis. Antimicrob. Agents Chemother. **38:**340–344.
8. **Falkiner, F. R.** 1992. *Enterobacter* in hospital. J. Hosp. Infect. **20:**137–140.
9. **Gaston, M. A.** 1987. *Enterobacter*: an emerging nosocomial pathogen. J. Hosp. Infect. **11:**197–208.
10. **Georghiou, P. R., R. J. Hamill, C. E. Wright, J. Versalovic, T. Koeuth, D. A. Watson, and J. R. Lupski.** 1995. Molecular epidemiology of infections due to *Enterobacter aerogenes*: identification of hospital outbreak-associated strains by molecular techniques. Clin. Infect. Dis. **20:**84–94.
11. **Grattard, F., B. Pozzetto, P. Berthelot, I. Rayet, A. Ros, B. Lauras, and O. G. Gaudin.** 1994. Arbitrarily primed PCR, ribotyping, and plasmid pattern analysis applied to investigation of a nosocomial outbreak due to *Enterobacter cloacae* in a neonatal intensive care unit. J. Clin. Microbiol. **32:**596–602.
12. **Grimont, F., and P. A. D. Grimont.** 1992. The genus *Enterobacter*, p. 2797–2815. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes, 2nd ed. Springer-Verlag, New York, N.Y.
13. **Grimont, P. A. D., M. Y. Popoff, F. Grimont, C. Coynault, and M. Lemelin.** 1980. Reproducibility and correlation study of three deoxyribonucleic acid hybridization procedures. Curr. Microbiol. **4:**325–330.
14. **Haertl, R., and G. Bandlow.** 1993. Epidemiological fingerprinting of *Enterobacter cloacae* by small-fragment restriction endonuclease analysis and pulsed-field gel electrophoresis of genomic restriction fragments. J. Clin. Microbiol. **31:**128–133.
15. **Lehn, N., J. Stöwer-Hoffman, T. Kott, C. Strassner, H. Wagner, M. Krönke, and W. Schneider-Brachert.** 1996. Characterization of clinical isolates of *Escherichia coli* showing high levels of fluoroquinolone resistance. J. Clin. Microbiol. **34:**597–602.
16. **National Committee for Clinical Laboratory Standards.** 1992. Performance standards for antimicrobial disk susceptibility tests. Publication no. M2A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
17. **O'Hara, C. M., A. G. Steigerwalt, B. C. Hill, J. J. Farmer III, G. R. Fanning, and D. J. Brenner.** 1989. *Enterobacter hormaechei*, a new species of the family *Enterobacteriaceae* formerly known as enteric group 75. J. Clin. Microbiol. **27:**2046–2049.
18. **Shlaes, D. M.** 1993. The clinical relevance of *Enterobacter* infections. Clin. Ther. **15:**21–28.
19. **Weinstein, R. A.** 1986. Endemic emergence of cephalosporin-resistant *Enterobacter*: relation to prior therapy. Infect. Control **7:**120–123.
20. **Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey.** 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:**6531–6535.
21. **Woods, C. R., Jr., J. Versalovic, T. Koeuth, and J. R. Lupski.** 1992. Analysis of relationships among isolates of *Citrobacter diversus* by using DNA fingerprints generated by repetitive sequence-based primers in the polymerase chain reaction. J. Clin. Microbiol. **30:**2921–2929.